Reference: *Biol. Bull.*, **147**: 247–256. (August, 1974)

THE PROCESS OF EGG-LAYING IN THE CHAETOGNATH *SAGITTA HISPIDA* [1]

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The process of egg-laying was described in *Sagitta bipunctata* by Boveri (1890), Stevens (1905, 1910), Bordàs (1920) and Ghirardelli (1968), in *S. hispida* by Conant (1896), in *S. hexaptera* by Grassi (1883), in *S. elegans* by Stevens (1905, 1910) in *S. setosa* by Dallot (1967, 1968), in *S. inflata* by Ghirardelli (1968), and in *Spadella cephaloptera* by Vasiljev (1925) and Ghirardelli (1968).

These authors do not agree as to how the ovum is fertilized by the sperm, from which it is separated by a layer of germinal epithelium known as the crescent. Disagreement also exists concerning the manner in which the fertilized egg reaches the exterior, the nature of the opening through which it leaves the body, and even on the number of ducts in the female reproductive system.

Reeve and Walter (1972a) described mating in *Sagitta hispida*, during which one or both spermatophores may be exchanged, following which the sperm migrate down the body of the animal to the nearest gonopore. Upon reaching it, they separate into two groups, one of which enters the adjacent gonopore while the other crosses over the body to the gonopore on the opposite side. The sperm enter the gonopores and move rapidly up a duct which lies on the outer side of each ovary.

The observations reported below describe events beyond this point which result in the laying of eggs, and indicate that in *Sagitta hispida*, at least, there is one tube which serves both as a sperm duct and an oviduct, and that the eggs are extruded by contractions of the ovary wall and body wall.

METHODS

Sagitta hispida Conant, a neritic species of chaetognath of approximately 10 mm mature length, is readily obtainable in plankton tows made from the laboratory dock on Biscayne Bay (Miami), and can be cultured in the laboratory for periods of several weeks. The detailed methodology of culture was provided by Reeve and Walter (1972b). In summary, animals were maintained in transparent acrylic 80-liter aquaria and fed natural zooplankton. The observations reported below were made on many such populations over a period of two years by withdrawing organisms from aquaria, placing them in small, shallow dishes or upon slides, and observing them live under the microscope. Of hundreds of animals examined in this way, numerous photomicrographs were made of organisms at various stages in the sexual cycle. In some cases individual animals were retained for several hours up to 2 days in 250-ml containers for further observation on the progress

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of egg-laying in the live animal. At least 30 animals, having been examined at various stages of egg-laying, were fixed in Bonin's solution, sectioned (8 μ) and stained with eosin and haematoxylin. This material was examined and photographed up to magnifications of × 1000. The external appearance of the gonopore region was also examined by scanning electron microscopy after preparation as described by Cosper and Reeve (1970). Observations on egg deposition were made on mature populations of chaetognaths in 80-liter aquaria into which had been transplanted three of the commonest local benthic plants (*Thalassia testudinum*, *Syringodium filiforme* and *Diplanthera wrightii*). The surfaces of the aquaria and of the plants (the latter having been removed to dishes) were examined for the presence of eggs after 48 hours.

Observations

Fertilization

Following mating, sperm can be seen in live animals examined microscopically (see figures in Reeve and Walter, 1972a) making vigorous movements in the duct running along the outer side of each ovary, and in prepared sections (Figs. 1A and B). Although the act of fertilization was not seen, at the cellular level, it appeared to be effected by passage of the sperm through a stalk consisting of two cells (Fig. 1A) which appear, in section, to be highly vacuolar, and somewhat indistinct at their boundaries, as though on the point of degenerating. The stalk, (accessory fertilization cells, see Discussion) is part of the germinal epithelium from which the immature ovum grows out, and through which it maintains an attachment to the epithelium as it develops.

Migration of mature eggs out of the ovary

The egg in Figure 1B is fully mature and about to migrate from ovary to duct; it is separated from the duct by only a thin strand of germinal epithelium.

In order to reach the duct, the egg must pass through the germinal epithelium. It appears to do this following degeneration of the stalk cells, or their being engulfed by the egg, by insinuating itself very gradually (Figs. 1C–F) through the resulting pore. First the egg becomes oval, rather than spherical, and then it becomes pear shaped. The small lobe of the pear is pushed through the pore and into the duct. Once through, it is gradually enlarged until the major part of the egg has migrated, the egg being somewhat dumb-bell shaped during this process. Then the remainder of the egg slips through, till the migration is complete.

This process seems to us to be accomplished by several means. The egg itself appears to make squeezing movements, though these are perhaps of too precise a nature to be termed ameboid. Also we have observed peristaltic waves of contractions passing along the ovary from anterior to posterior together with the appearance of bands circumscribing the ovary wall (Fig. 1G). Possibly these contractions help to propel the eggs through the crescent. In addition, pressure of the gut and even the normal muscular contractions of swimming movements may aid the eggs in their migration. The time required for all the mature eggs to complete their migrations, which are performed randomly rather than in unison, varies from 15 to 20 minutes.

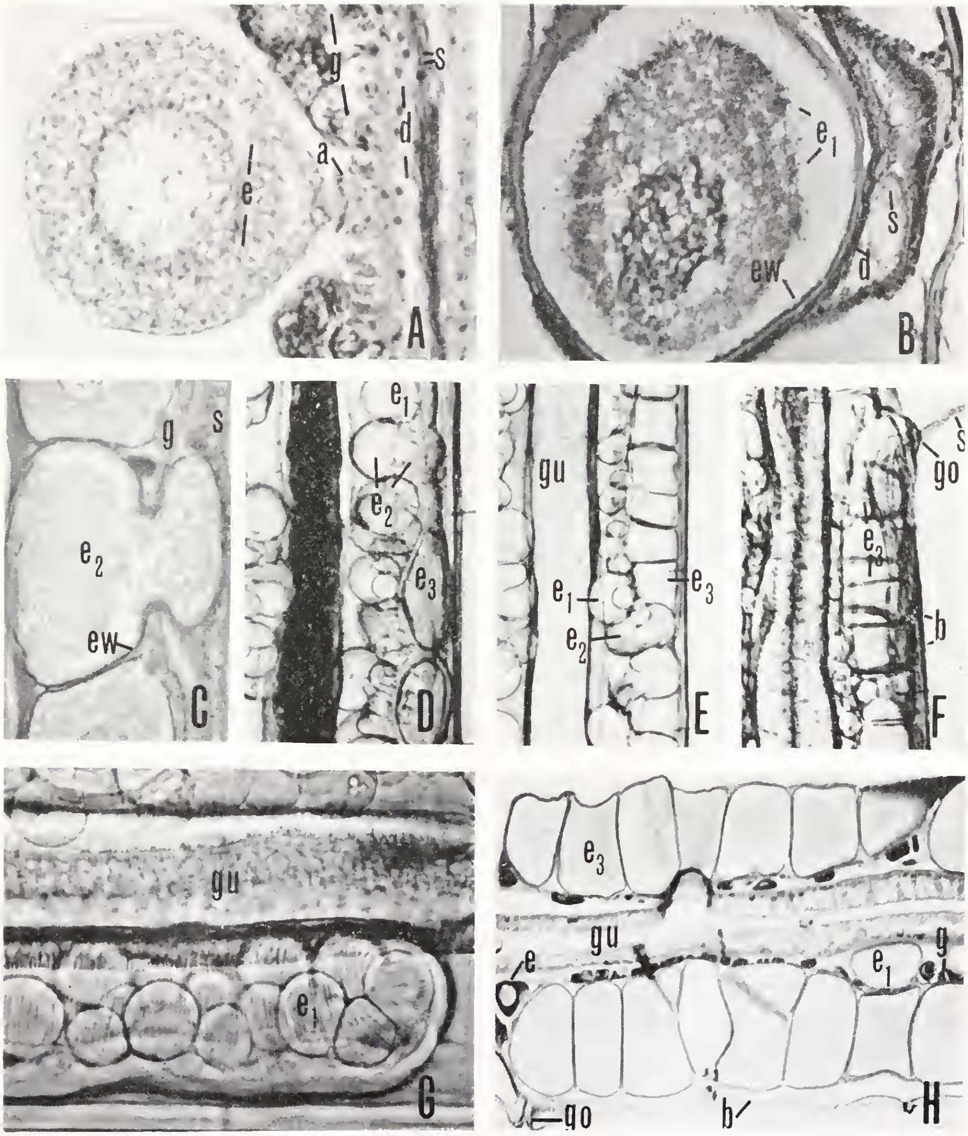

FIGURE 1. A—maturing egg; B—mature egg; C to F—stages in migration of egg to duct; G—ovary with mature unmigrated eggs showing contractions of ovary walls; H—eggs in duct immediately prior to laying. Figures A–C and H are from sections, D–G from live material; width of body approximately 0.5 mm; a—accessory fertilization cells; b—body wall; d—duct; e—maturing egg; e_1—mature unmigrated egg; e_2—migrating egg; e_3—migrated egg; cw—egg cell wall; g—germinal epithelium; go—gonopore; gu—gut; s—sperm.

As more and more eggs pass through the germinal epithelium into the duct, they become crowded (Figs. 1D, E and F), possibly because the lumen of the duct is narrower than the diameter of the eggs. They are compressed, as Conant (1896; page 82) described it, "from before backward" until their shape is cylindrical rather than spherical. When all the eggs have migrated, the duct is completely filled with eggs (Fig. 1H) so that, viewed from above, it has the appearance of a ladder, the boundaries between two adjacent eggs froming the rungs, and the walls of the duct and the outer boundaries of the eggs forming the uprights of the ladder. The immature eggs in the ovary are compressed against the gut (Figs. 1H and 2B). The animal will now lay its eggs within 10 or 15 minutes. In sections of individuals fixed at this stage there is no evidence of any duct other than the one occupied by the eggs (Figs. 1H and 2B).

Animals which are almost ready to lay eggs often attach to the side walls of the aquarium but they do not necessarily remain continuously attached till the laying is accomplished, but rather shift position every few minutes. Even animals whose eggs are migrating tend to move sporadically about, and we suggest that the muscular contractions of the body wall may aid in compressing the ovaries so that the eggs may more easily migrate from ovary to duct.

The duct

In sections of inseminated animals whose eggs have already migrated through the germinal epithelium, sperm may be seen directly adjacent to the cell wall of the egg (Figs. 2B and C). In addition to the direct visual evidence that sperm are not separated from eggs when the latter emerge on the outer side of the crescent, at 1000 magnifications, even under low power sperm bulges can be seen where they are trapped between two adjacent eggs or forced anteriorly into the blind end of the duct. Indeed, it is not unusual to see bundles of motile sperm extruded to the exterior (Fig. 1F) as eggs migrate through the crescent. There is, therefore, no evidence that eggs and sperm occupy different ducts.

The gonopore

Observations of the gonopore, both in sections (Fig. 2B) and with the scanning electron microscope, indicate that it has only one opening, and that only one duct connects with it. Sections of animals in which eggs have migrated prior to being laid (Figs. 1H, 2A and B) clearly show the terminal egg in the cavity of a duct continuous with the opening of the gonopore, and where sperm are present (Fig. 2B) show them to occupy the same cavity.

Expulsion of mature eggs

Our observations indicate that the eggs are expelled by a sudden and intense contraction of the ovary and body walls. We are indebted to our colleague, M. A. Walter, for a photograph of a live animal (Fig. 2D) in the process of expelling its eggs, in which the body wall contractions are clearly visible. Contractions of the ventral body-wall musculature in the region of the gonopores (Fig. 2A) is presumably also involved, by expanding the orifice of the gonopores so that the eggs

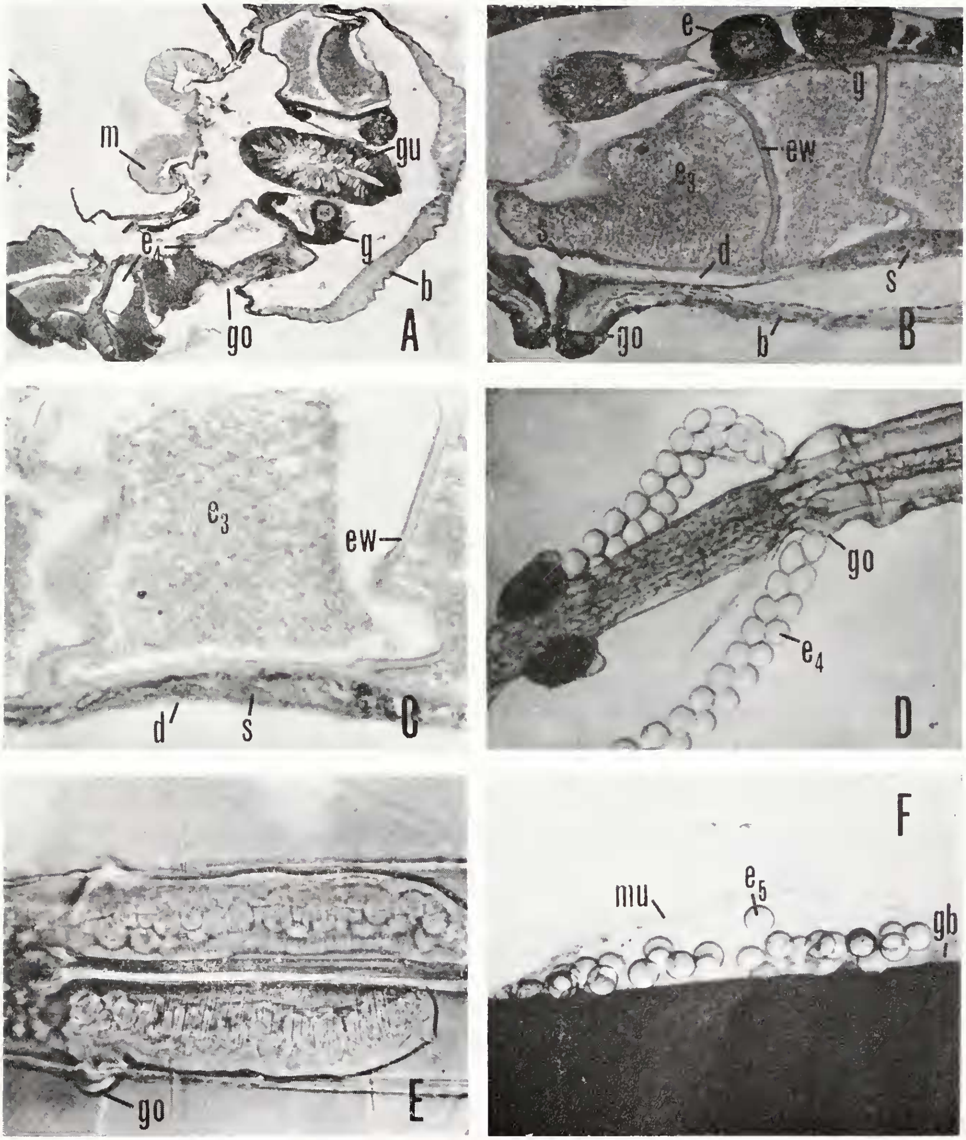

FIGURE 2. A—eggs in process of being laid from gonopore; B—eggs about to be laid adjacent to gonopore; C—sperm and eggs in duct; D—eggs being laid; E—ovary showing contraction bands after egg-laying; F—mucus-embedded eggs attached to grass blade. Figures A–C are from sections, D–F from live material. Diameter of eggs approx. 0.2 mm. e_4—eggs in process of being laid; e_5—attached (laid) eggs; gb—grass blade; m—muscle; mu—mucus, Other abbreviations as in Figure 1.

do not have to deform their shape (Fig. 2A) as much as they did in order to pass through the crescent.

The eggs issue in two streams from each gonopore (Fig. 2D), and not, as Conant (1896) reported, one from each gonopore, so that the egg mass ultimately has the appearance of four strands of beads. The eggs are laid about ten or fifteen minutes after the last one has migrated through the germinal epithelium (at 26° C).

Following laying, the ovaries appear crumpled and contracted in an anterior-posterior plane; they are wide, filling the diameter of the body cavity but not even extending as far forward as the anterior of the posterior fin (Fig. 2E). They may be distinguished from those of a less mature animal which has yet to lay eggs (see Reeve and Cosper, 1974), for in the latter the ovaries are narrow and smooth-walled. As noted elsewhere (Reeve and Cosper, 1974) *Sagitta hispida* does not shed its eggs into the water column. In the laboratory strings or clumps of eggs attach as they emerge to the side walls of the aquarium, or to the bottom in the case of small dishes. When three common representatives of the local benthic macroflora (*Thalassia testudinum*, *Syringodium filiforme* and *Diplanthera wrightii*) were placed in aquaria with mature chaetognaths, we observed that the animals would attach to and deposit their eggs on the vegetation (Fig. 2F) provided that the latter was securely anchored to the bottom of the container.

Discussion

In order to account for the method by which the sperm reaches to ovum, Buchner (1910) and Stevens (1903, 1905) proposed the existence of two accessory fertilization cells, the outermost being inserted in the wall of the "oviduct" and the inner being inserted in the ovum. The sperm swims through a series of vacuoles in these cells from the "oviduct" to the ovum. Ghiradelli (1968) confirmed these observations in *Sagitta bipunctata* and *Spadella cephaloptera*.

Our observations confirm those of Conant (1896) who suggested that the developing oocytes are attached to the ovary by a stalk (Fig. 1A) and contradict those of Stevens (1903) who maintained that the eggs develop free inside the ovary and then "each one becomes connected with two of the epithelial cells which are just lateral to the region" (page 232). This stalk later serves as a passage for the sperm to the egg as suggested by Stevens (1903), Buchner (1910), Vasiljev (1925) and Ghirardelli (1968).

Following fertilization, and indeed, even in its absence (Reeve and Cosper, 1974) the mature eggs move from the ovary into the duct which leads to the gonopore. Since they make this migration irrespective of whether copulation and fertilization has occurred, this process must be triggered by the state of maturation of the egg itself and not by its penetration by the sperm.

In *Sagitta* the egg must pass through the germinal epithelium of the crescent into the duct by which it gains access to the exterior. Exactly how the egg moves, or is moved, is uncertain, although Dallot (1968) has reported seeing contractions of the ovary wall, confirming Conant's (1896) earlier observations. Ghirardelli (1968) and Stevens (1905, 1910), however, maintained that the egg makes its way into the duct by ameboid movements. In *Sagitta hispida* the movement from ovary to duct appears to be partly active and partly by pressure exerted by the ovary walls and possibly other tissues. In *Spadella*, Ghiradelli (1968) indicated that the eggs moved posteriorly in the ovary before entering a very short terminal duct.

Perhaps the greatest source of controversy concerns the structure of the female reproductive organs, and in particular the nature and position of the duct which the egg enters on leaving the ovary. Hertwig (1880) wrote that the ovaries consisted of the ovary or egg tube and the oviduct. Conant (1896), working with *Sagitta hispida,* suggested that there was an oviduct (separate from the sperm duct) lying between the germinal epithelium and the epithelium of the sperm duct. According to his observations, after the eggs were fertilized, they moved through the germinal epithelium into the oviduct, which was a temporary structure.

Stevens (1910) in studying *S. bipunctata,* stated that "both living material and sections show that the sperm duct and oviduct are entirely separate for their whole length, each having its own opening to the exterior" (page 284). She confirmed Conant's (1896) observations concerning the temporary oviduct.

Later authors fell into one or other of the two camps. Bordàs (1920), while working on the same species studied by Stevens, stated that the single tube, although serving as a sperm duct, is principally an oviduct.

Vasiljev (1925) referred to a sperm duct and mentioned elastic fibers running along the length of this duct, which, he said, serve to keep the sperm duct extended. He was unable to find anything resembling a provisional or temporary oviduct, and stated that in *Spadella* the eggs are liberated from the body of the adult animal by a rupture of the body wall occurring close to the entrance of the sperm duct. He also suggested that the accessory fertilization cells and sperm duct degenerated after having fulfilled their role.

Burfield (1927) described the sperm duct and temporary oviduct in detail. He said that the outer duct (oviduct) surrounds the sperm duct which is merely a sperm pouch, having syncytial walls. He did not, however, describe the two openings to the exterior which two such ducts would necessitate.

John (1933), studying *Spadella cephaloptera* and a species of *Sagitta,* confirmed Stevens' (1905, 1910) observations concerning the movement of the eggs, but stated that, in *Spadella,* there was a direct opening between the ovary and the seminal receptacle; however, this orifice was visible only at the time of laying.

John (1943) later had occasion to study *Sagitta* sp. further, and reversed his earlier opinion concerning the presence of two ducts. He stated that there is only one duct, and that what was previously thought to be a second internal duct with syncytial walls was merely "rounded masses of degenerate sperms . . ." (page 71) which would ultimately be reabsorbed.

Dallot (1968) referred to a provisional oviduct, and Ghirardelli (1968), while confirming the observations of Stevens (1910) on *Sagitta bipunctata,* continued "Studies *in vivo* do not allow firm conclusions as to how the eggs reached the outside: only the study of sections obtained from specimens fixed in the various phases of laying has enabled Stevens to describe for the first time the passage of the eggs to the exterior. I have been able to confirm her observations and show the first photographic evidence of the phenomenon" (page 336). Ghirardelli's photographic evidence is of a section however, and shows an egg passing through the crescent: it does not show how the eggs are laid.

While it is possible, and even probable, that differences do indeed exist between one species of chaetognath and another, the above review indicates differences of opinion concerning the same species. Concerning *S. bipunctata,* Bordàs (1920) acknowledged the existence of one duct, whereas Stevens (1905, 1910) was able to see two. In *Spadella cephaloptera,* Vasiljev (1925) mentioned that there is a sperm duct and a temporary oviduct, though he was unable to see the latter, and that the sperm duct is also a temporary structure, while Ghirardelli (1968) indicated that only the oviduct is of a temporary nature. The majority of workers support Conant's (1896) theory that there are two ducts in chaetognaths, and oviduct of a temporary nature, and a permanent sperm duct. However, our observations on *Sagitta hispida* (the species with which Conant, 1896, worked) indicate that there is only one duct, which serves for the admission of the sperm and for the expulsion of the eggs. Apart from our direct visual observations on the direct contact of eggs and sperm within a single duct, we saw extrusion of sperm to the exterior as eggs migrated through the crescent. It seems highly likely that the sperm are being forced into these situations by displacement of the eggs as they enter the same duct occupied by the sperm, although it could be argued that the same effect would be seen in an adjacent tube which was compressed. Sperm which are extruded, however, leave the body via the gonopore (Figs. 1F and 2B) exactly as do the eggs. Further, Reeve and Walter (1972a) showed that insemination confers fertility on one batch of eggs only, subsequent eggs being infertile when laid, if the animal is prevented from being reinseminated. It would seem that a primary advantage of a separate sperm duct would be to store viable sperm for fertilization of ova which had yet to mature.

Stevens (1910) suggested that eggs reached the exterior by their own active movements, crawling along the duct to the gonopore. Ameboid movements were also observed by John (1933) and Ghirardelli (1968). Conant (1896) described contractions of the ovary wall (which surrounds the developing ova, germinal epithelium and duct) as providing the force for the expulsion of eggs in *Sagitta hispida* and Dallot (1968) confirmed this for *S. setosa.* Grassi (1883), Stevens (1910) and Vasiljev (1925) had noted the presence of elastic fibers. Our observations showed that the eggs, which become cylindrically compressed in the duct, are extruded very rapidly by a combination of muscular forces, which includes the body wall musculature as well as the ovary itself.

In *Sagitta hispida,* we observed, as already noted, that eggs leave the body via the single ovarian duct through the gonopore. Regarding other members of the genus, Vasiljev (1925) believed that eggs left the temporary oviduct by being extruded through a rupture in the body wall. From the figures of Ghirardelli (1968) it appears that a temporary opening to the oviduct occurs adjacent to the opening of the sperm duct at the gonopore. In *Spadella,* Ghirardelli (1968) indicated by description and a figure that eggs pass down to the posterior end of the ovary before entering a short temporary oviduct formed by a splitting of the two layers of the wall of the terminal portion of the sperm duct, and exit by a temporary opening to the exterior. John (1933) had previously indicated that the eggs penetrated into the seminal receptacle (the expanded portion of the sperm duct at the gonopore).

Sagitta hispida is a neritic species (Owre, 1960) and is the only planktonic chaetognath which has adapted to the semi-estuarine conditions of Biscayne Bay. The behavioral pattern of attaching eggs presumably assists in maintaining the Biscayne Bay population, since that part of the life cycle during which either the mature adult or the egg is attached is less susceptible to expatriation (an inherent danger of a planktonic existence) into the Florida Current adjacent to Biscayne Bay.

This work was supported by National Science Foundation Grant number GA-28522 (Oceanography Section).

Summary

1. There is considerable disagreement in the literature concerning the manner in which eggs are fertilized and reach the exterior in chaetognaths, even in studies on the same species. This study makes use of observations on populations of living animals maintained in the laboratory, and of material fixed at known stages of the egg-laying process, and provides photographic evidence to illustrate the points enumerated below.

2. There is a single duct, serving both as an oviduct and sperm duct in the female reproductive system of the chaetognath *Sagitta hispida,* which runs from the gonopore anteriorly along the outer border of each ovary.

3. Following fertilization eggs slowly pass through the germinal epithelium (crescent) through pores much smaller than their own diameter into this duct, and displace the sperm in it.

4. The eggs, which become cylindrically compressed, are extruded very rapidly from the duct to the exterior by a combination of muscular forces, and deposited in a mucous matrix onto a surface to which the animal is attached.

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